

CLAIMS

We Claim:

1. A method for detecting RNA-dependent polymerase activity comprising:
 - (a) providing a primer oligonucleotide having a 3' OH;
 - 5 (b) contacting said primer oligonucleotide with a template polynucleotide and allowing hybridization to occur to form a hybridized polynucleotide;
 - (c) adding an RNA-dependent polymerase to said hybridized polynucleotide to produce a mixture;
 - (d) adding a PP_i detection mixture to said mixture;
 - 10 (e) adding a substrate mixture comprising a nucleotide triphosphate or an analog thereof to said mixture; and
 - (f) measuring a product of the PP_i detection mixture;wherein
 - aprase is not part of the mixture and
 - 15 steps (c), (d) and (e) may be performed simultaneously or separately in any order.
2. The method of claim 1, wherein said RNA-dependent polymerase is a viral RNA-dependent RNA polymerase (RdRp) selected from the group
 - 20 consisting of Hepatitis C virus, poliovirus, West Nile virus, Dengue virus, Human T Cell Leukemia virus, St. Louis Encephalitis virus, Yellow Fever virus and Measles virus.
3. The method of claim 2, wherein said RdRp is from Hepatitis C virus.
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4. The method of claim 1, wherein said RNA-dependent polymerase is an RNA-dependent DNA polymerase (RdDp).
5. The method of claim 4, wherein said RdDp is reverse transcriptase from
 - 30 Human Immunodeficiency virus.

6. The method of claim 1, wherein said hybridized polynucleotide comprises synthetic poly(A) and poly(U).
7. The method of claim 1, wherein said hybridized polynucleotide
5 comprises synthetic poly(G) and poly(C).
8. The method claim 1, wherein said primer oligonucleotide and said template polynucleotide are on the same RNA molecule.
- 10 9. The method of claim 1, wherein said PP_i detection mixture comprises luciferase, luciferin, ATP sulphurylase and adenosine 5'-phosphosulfate (APS) and said product is emitted light.
- 15 10. The method of claim 9, wherein the emitted light is measured with a luminometer.
11. The method of claim 9, wherein said luciferase is a thermostable luciferase.
- 20 12. A method for evaluating an inhibitor of an RNA-dependent polymerase comprising:
 - (a) providing a primer oligonucleotide having a 3' OH;
 - (b) contacting said primer oligonucleotide with a template polynucleotide and allowing hybridization to occur to form a hybridized polynucleotide;
 - 25 (c) adding an RNA-dependent polymerase to said hybridized polynucleotide to produce a mixture;
 - (d) adding a PP_i detection mixture to said mixture;
 - (e) adding a substrate mixture comprising a nucleotide triphosphate or an analog thereof to said mixture;
 - 30 (f) adding a compound that is or is suspected of being an inhibitor of said RNA-dependent polymerase; and
 - (g) measuring a product of the PP_i detection mixture;

wherein

aprase is not part of the mixture, and

steps (c), (d), (e) and (f) may be performed simultaneously or separately in any order.

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13. The method of claim 12, wherein said RNA-dependent polymerase is a viral RNA-dependent RNA polymerase (RdRp) selected from the group consisting of Hepatitis C virus, poliovirus, West Nile virus, Dengue virus, Human T Cell Leukemia virus, St. Louis Encephalitis virus, Yellow Fever virus and Measles virus.

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14. The method of claim 13, wherein said RdRp is a recombinantly produced Hepatitis C virus NS5B.

15 15. The method of claim 12, wherein said RNA-dependent polymerase is an RNA-dependent DNA polymerase (RdDp).

16. The method of claim 15, wherein said RdDp is reverse transcriptase from Human Immunodeficiency virus.

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17. The method of claim 12, wherein said hybridized polynucleotide comprises synthetic poly(A) and poly(U).

18. The method of claim 12, wherein said hybridized polynucleotide comprises synthetic poly(G) and poly(C).

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19. The method claim 12, wherein said primer oligonucleotide and said template polynucleotide are on the same RNA molecule.

30 20. The method of claim 12, wherein said PP_i detection mixture comprises luciferase, luciferin, ATP sulphurylase and adenosine 5'-phosphosulfate (APS) and said product is emitted light.

21. The method of claim 20, wherein the emitted light is measured with a luminometer.
22. The method of claim 21, wherein said luciferase is a thermostable
5 luciferase.